NORMAL HISTOLOGY OF THE KIDNEYS, LIVER, HEART, SPLEEN, LUNG OF THE GUINEA PIG AND HISTO-PATHOLOGICAL CHANGES OF THESE ORGANS IN EXPERIMENTALLY INDUCED BOTULISM

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED NORMAL HISTOLOGY OF THE KIDNEYS, LIVER, HEART, SPLEEN, LUNG OF THE GUINEA PIG AND HISTO-PATHOLOGICAL CHANGES OF THESE ORGANS IN EXPERIMENTALLY INDUCED BOTULISM.

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

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The sporadic outbreaks of botulism in animals reported in recent years has suggested the desirability of accurate diagnostic methods. In a peracute attack of the atypical type of this disease the symptoms are not pathognomonic, and are probably frequently confused with other diseases. The presence of the spore in the intestinal tract, or in the internal organs as an agonal invasion following death, is not irrefutable diagnostic evidence of botulism. The microscopic changes in the tissues of the guinea pig, after experimentally inducing botulism, have been studied with the view of determining, if possible, the value of histo-pathological changes in the kidneys, liver, heart, spleen and lungs. In the following paper a brief study of the histology of the normal guinea pig and a study of tissues obtained from cases of experimentally induced botulism are recorded.

METHOD OF FIXING, EMBEDDING, CUTTING AND STAINING OF TISSUES

Fixing and Embedding Tissues:

It is essential in the sectioning of body tissues to retain the normal relationship of the cells of the organ as present in the individual. Post mortem changes take place rapidly so that the tissues should be placed in a fixing fluid (10 % Formalin) as soon as possible. After the fixation is complete the tissues are washed and later dehydrated by placing them in a series of solutions of increasing concentrations of alcohol.

Prior to the process of embedding, the tissues are cleared in xylene, and after being completely infiltrated with hot paraffin, they are embedded in blocks of the same material. The following technic was employed in fixing, embedding, cutting and staining the preparations. The tissues for sectioning purposes were cut about two centimeters square and after being washed free of excess blood they were placed in a volume of the 10 % formalin solution about fifty times the volume of the tissue.

Fixing and Embedding Technic:

10 % Formalin in physiologic salt solution for 24 hrs.
Wash for 24 hrs.
70 % Alcohol 24 hrs (or longer).
95 % Alcohol 24 hrs.
Absolute Alcohol 24 hrs.
Xylene 2 hrs.
Xylene-Paraffin Mixture***--2 hrs)--In oven at 58°C.
Paraffin-Beeswax Mixture***-4 hrs)
Embed in Paraffin-Beeswax Mixture

The tissues are embedded in paper boats, which are of common white paper about eight centimeters long and three centimeters wide. Each boat is placed upon a cold surface and filled half full of the warm paraffin-beeswax mixture. Then with warm forceps the square of tissue is placed in the boat, the surface to be cut being embedded down. The filling of the boat is completed with the warm paraffin. This second pouring will drive out bubbles of air which adhere to the bottom. The boat is then floated in cold water to speed the solidification of the paraffin and drive out air.

***See preparation on page 4.

Cutting of Microscopic Sections:

The paper boats are stripped from the hardened paraffin and the blocks trimmed, taking care that the edges of the cutting surface are parallel to insure ribboning of the sections. A horizontal sliding microtome was used in this project. Four microns was the usual thickness of the sections, but with certain blocks it was only possible to get good sections at five or six microns. The cut sections were floated on warm water and mounted on a slide by putting the slide underneath the floating section and raising it out of the water. The sections were held to the slide by a Japanese glycerine-albumin mixture** previously rubbed on the slide and heated until a steam began to arise from the surface. The mounted sections were allowed to dry in an oven at 58°C for 12 hours.

Staining of the Sections:

Hematoxylin and eosin were used for staining all tissues. The hematoxylin stains the nuclei purple and the cytoplasm takes the eosin or acid stain.

Preparation of Delafield's Hematoxylin.

Hematoxylin crystals-------4 grams
Alcohol (absolute)--------25 cc.
Saturated solution of
ammonium alum-400 cc.

The hematoxylin crystals are dissolved in the absolute alcohol. To this solution 400 cc of alum solution was added. The mixture was exposed to the light (sunlight if possible) in an unstoppered bottle for 3 to 4 days. After filtration the following were added:

Glycerine----------100 cc
Alcohol (absolute)--------100 cc

This solution was allowed to stand in the light until the color was sufficiently dark, then filtered and kept in a tightly stoppered bottle. The solution keeps well and is satisfactory as long as it retains a purplish tinge.

Preparation of Eosin Stain:

Eosin----------1 gram
Water----------100 cc
Absolute alcohol----------15 cc

The eosin was dissolved in the distilled water, the alcohol added, and filtered.

**Preparation on page 4.

The staining was accomplished by the following technic:

Xylene----------3 min.
Absolute Alcohol------2 min.
95% Alcohol----------2 min.
60% Alcohol----------2 min.
Distilled H_2O---------2 min.
Hematoxylin-------6to30 min.
Tap water----------2 min.
Distilled H_2O---------2 min.
Eosin----------2 to 3 min.
Tap water----------2 min.
Distilled H_2O---------2 min.
70% Alcohol----------1 min.
95% Alcohol----------3 min.
Absolute Alcohol------4 min.

All excess staining matter on the slide around the tissue was removed with a cloth.

Xylene----------3 min.

The surplus xylene was cleaned off and a drop of Canadian balsam added to the section over which a cover slip was then gently placed taking care to exclude all air bubbles. A light weight was placed on the cover slip to hold it in place until the balsam dried. The slides were set aside to allow the balsam to dry and seal the cover slip.

Preparation of Xylene-Paraffin Mixture:

1 part 56°C paraffin
1 part 46°C paraffin
2 parts xylene

Slowly melt the paraffin and add the xylene to the liquid paraffin.

Preparation of Paraffin-Beeswax Mixture

Paraffin 850 grams.
Stearin 100 grams
Beeswax 50 grams

This mixture is kept in the oven at 56°C.

Preparation of Japanese Glycerin-Albumin.

White of Egg)
)--Equal parts
Glycerine)

Beat mixture and filter. Add a few drops of formalin as a preservative.

SOURCE OF MATERIALS

The normal tissue specimens which were used for comparative purposes

were obtained by destroying a healthy guinea pig and immediately removing the fresh organs. The guinea pigs furnishing the pathological tissue specimens were originally used in botulinus typing tests. A majority of the guinea pigs (18) died in a few hours (12-30 hrs) following an acute attack of botulism, while two pigs were destroyed in the agonal stages of the disease in order to avoid the development of post-mortem changes.

KIDNEYS

Normal Histology:

The kidneys are glandular organs located on each side of the dorsomedian line. They are invested in a fibrous capsule which blends into the ureter at the hilum. The organ is divided into four regions, the cortex, medulla, intermediate zone, and pelvis. Extending into the sinus at the hilum are the renal papillae, the projections of the renal pyramids. These renal pyramids extend back thru the medulla and have their base towards the cortex. Between the pyramids are found the columns of Bertini which exterd to the walls of the sinus.

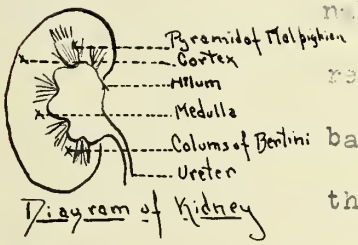
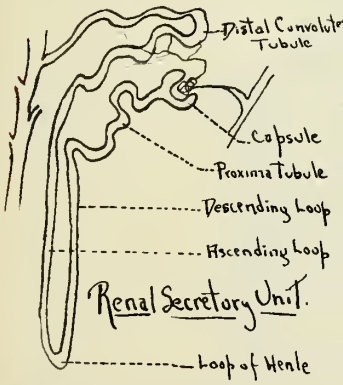


Diagram of Kidney

On the examination of the cortex under low power magnification it is seen that it is divided into alternating light and dark areas, the medullary rays and the labyrinth respectively.



Renal Secretory Unit.

The structure of a renal secretory unit is composed of three general parts namely, the glomerulus, the convoluted uriniferous tubules and the collecting tubules or duct system. As shown in the figure the uriniferous tubules begin in a greatly expanded blind extremity, the capsule. This capsule surrounds the tuft of an artery forming the Malpighian body. The tubule coming from the glomerulus takes a very tortuous and twisted course forming the proximal convoluted tubule. It then extends into the medullary rays and follows a straight course towards the papillae, as the descending limb of Henle. When well into the medullary region it turns upon itself, the loop of

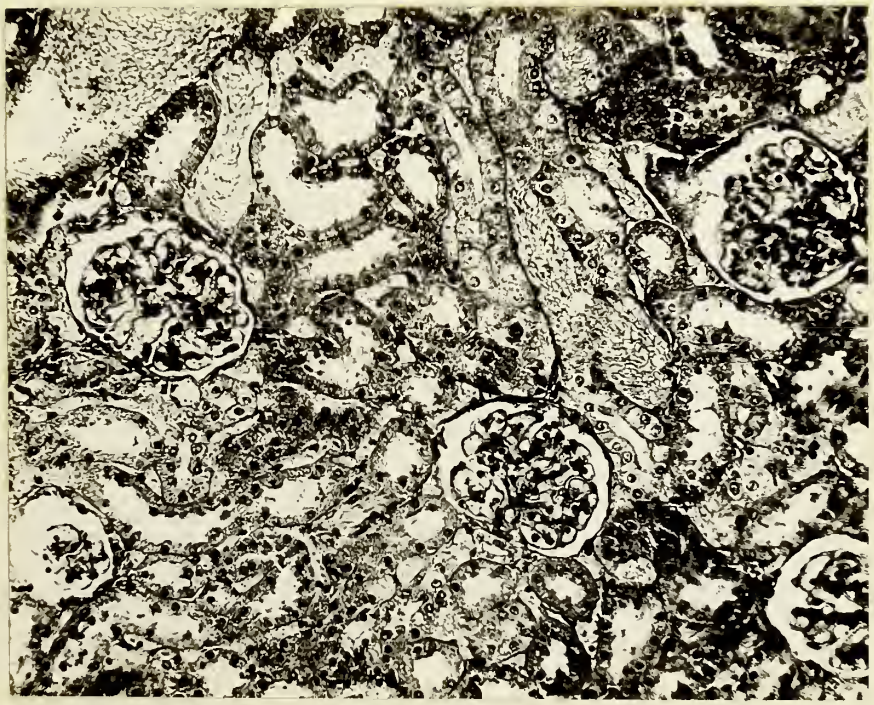
Henle, and returns to the renal pyramid and the medullary rays as the ascending limb of Henle. It again enters the labyrinth and repeats its tortuous convoluted course, forming the distal convoluted tubule. Later it reenters the medullary rays, and joining one of the collecting ducts, it finds its way into the renal pyramid and out through the papillae into the sinus.

The structure of the urinary tubules is very simple, consisting of a single layer of cells supported by a basement membrane. The glomerular portion eliminates the water and inorganic constituents, while the organic matter is eliminated thru the tubules. By this arrangement the tubules are continually washed by the liquid which carries the secretory products down the tubule. The urine collects in the sinus which has an outlet thru the ureter.

KIDNEY

Histo-Pathological Changes:

Parenchymatous degeneration of the urinary tubules was the most constant characteristic pathological change found in the kidneys. The degree of degeneration varied from a very severe one bordering on necrosis to a very mild degenerative disturbance. In the majority of the cases the parenchymatous degeneration was marked.



Kidney--Histopathological Changes

Passive congestion existed in over 80% of the cases. Over half of the series (19 cases) presented advanced passive congestion; the congestion backing up to involve the glomeruli. The passive congestion which involved the glomeruli was classified as severe, while the less severe cases were rated as marked and slight. Ten percent of the kidneys were markedly congested, while 30% were only slightly congested. A final 10% showed no passive congestion.

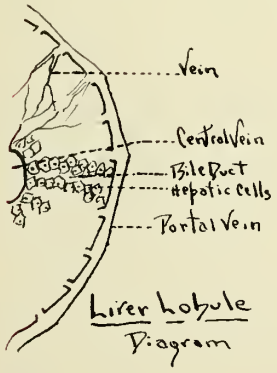
There were also other changes present in a few instances. A few hemorrhages were found in three cases. One kidney showed a beginning necrosis of the urinary tubules around the hemorrhagic area. Another organ also presented a few small areas of necrosis and one showed a slight edema in the cortex region.

In general the most constant changes were a marked parenchymatous degeneration and a marked passive congestion.

LIVER

The Normal Histology:

The liver, the largest gland in the body, consists of very delicate glandular tissue disposed around ramifications of the portal vein. It is covered with a capsule of fibrous tissue giving off trabeculae which extend into the interior. The position of the liver in the body cavity is immediately below the diaphragm chiefly on the right side with the smaller lobe extending into the left side of the cavity. Grossly the liver is divided into two large lobes. Each of these lobes is subdivided by the interlobular tissue into many lobules. Centrally located in each lobule, the central vein receives the capillary branches of the portal vein.



Liver Lobule Diagram

The hepatic cells, the smallest unit of structure of the liver, are polygonal in shape and contain a comparatively large nucleus. The cytoplasm is finely gramular, and the nucleus contains a reticularly arranged mass of chromatin material. The liver cells are placed in rows or cords projecting from the

central vein-like spokes in a wheel.

The biliary sinuses between the rows of cells which collect the secreted bile from the liver cells have no definite vessel walls but their course follows thru trough-like depressions in the walls of the liver cells. The larger bile vessels have true walls and are usually found in close proximity to a hepatic vein and a hepatic artery forming the so-called hepatic trinity.

Two blood supplies, namely, the functional and the nutritional, are found in the liver. The functional supply comes from the portal vein. After leaving the portal vein the blood flows into the interlobular branches of the vein which carry it thruout the liver and brings it to the individual lobules. These interlobular veins then give off capillary ramifications which extend thruout the lobule and bring the blood in intimate contact with the hepatic cells. Later the capillaries collect at the central vein and conduct the functional blood to the sublobular branch of the hepatic vein and finally back to the heart. The amount of interstitial tissue varies in different species. In swine the lobules are very distinct due to the large amount of interlobular connective tissue.

The nutritional blood is derived from the hepatic artery and follows a course somewhat similar to the functional blood. Later the two unite and both return to the heart by way of the same vessels.

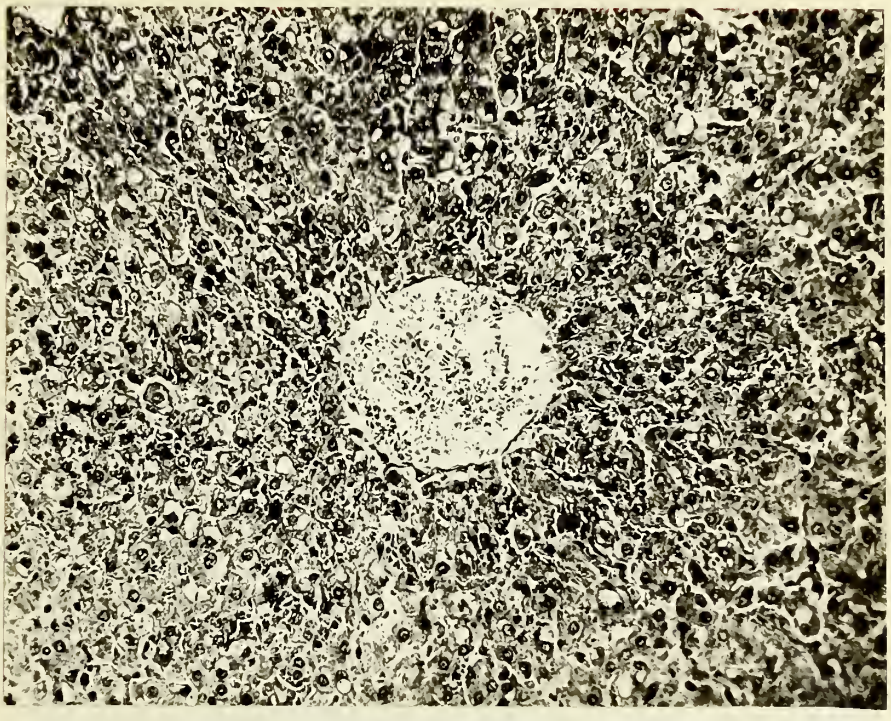
LIVER

Histo-Pathological Changes:

Passive congestion, tho not present in all the livers was characteristic of them in general. Two livers were severely congested, two others showed marked passive congestion, and ten were only slightly congested while five presented no noticeable congestion. There were no hemorrhages in any of this series (19 cases).

The most characteristic pathological changes in the livers were fatty degeneration and fatty infiltration. Only two cases could be classed as a pure fatty infiltration while fifteen cases showed fatty degeneration with some fatty

infiltration in three cases. It was interesting to note that there were only two cases which were free of pathological fat.



Liver--Histo-Pathological Changes

Sixteen of the guinea pigs showed marked parenchymatous degeneration of the liver. Most of the parenchymatous degeneration was present with fatty degeneration, while there were only two mild cases of parenchymatous degeneration in livers which did not show fatty degeneration. All cases showed either a parenchymatous or fatty degeneration. A slight cellular hepatitis was found in four cases.

Passive congestion, marked fatty degeneration, and parenchymatous degeneration were the three most characteristic pathological changes in the liver.

HEART

Normal Histology:

Histologically the heart is composed of three layers of tissue, the epicardium, myocardium and endocardium. The endocardium is composed of a single layer of endothelial plate-like cells and underlying connective tissue which is quite rich in elastic fibers with a small quantity of involuntary muscle. This layer is composed chiefly of muscle fibers which are not organized into bundles,

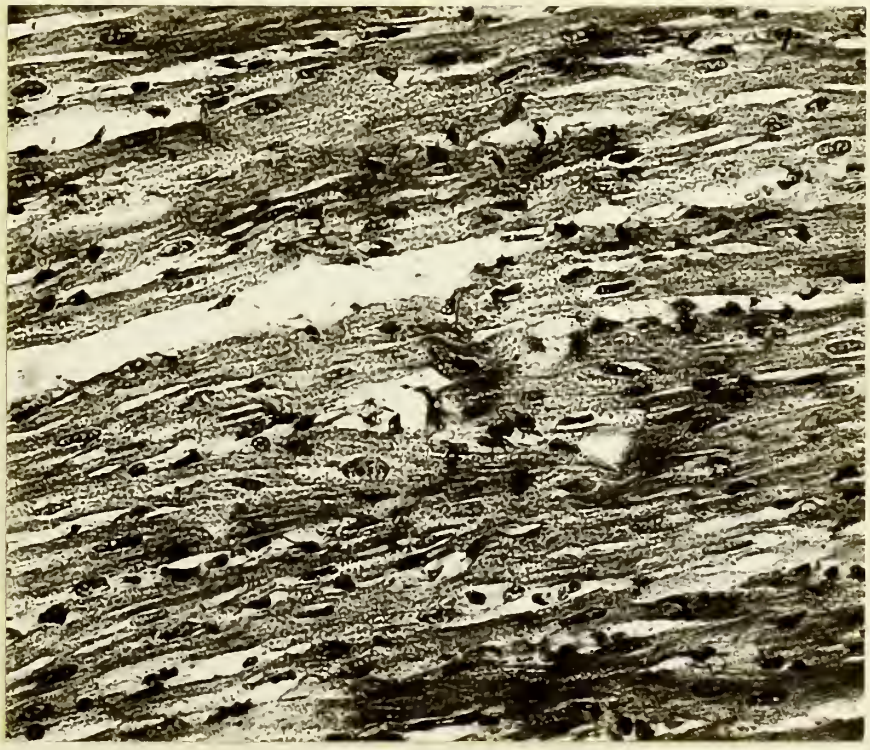
but form a very complex system of branching and intermingling fibers or intercalated discs. Between the branching cardiac muscle structure the connective tissue aids in binding the fibers together. There is no well defined endomysium as in voluntary muscle.

The outer layer of the heart, the epicardium, consists of a single layer of endothelial cells having a substratum of fibro-elastic tissue. The epicardium completely covers the heart and blends into the adventitia of the great veins.

HEART

Histo-Pathological Changes:

The only constant pathological change in the heart was a parenchymatous degeneration. Most of the heart series (16 cases) were only slightly degenerated, while two showed marked degeneration, and one severe degeneration.



Heart--Histo-Pathological Changes

The striations of the muscle fibers were not visible due to the parenchymatous degeneration. Congested coronary vessels were found in six specimens of this organ and one showed a passive congestion of the capillaries of the cardiac muscle. Another change present in three of the hearts, which might have been due

to the method of fixation, was a separation of the muscle fibers at the cement lines. The fibers of one of the hearts appeared as tho they were shrunken.

Summarizing the series of the heart it was found that parenchymatous degeneration was characteristic thruout and a congestion of the coronary vessels in a few cases.

THE SPLEEN

Normal Histology:

The spleen may be considered as a huge hemolymph node, possessing the functions of producing lymphocytes and of destroying erythrocytes. It is dark red in color due to the fact that it contains a large quantity of blood. This organ is located on the left side of the abdominal wall between the stomach and the diaphragm. The spleen is invested in a capsule composed of dense elastic tissue with a few scattered fibers of involuntary muscle. Extending from the capsule into the spleen are the trabeculae which branch into a delicate frame-work giving support and a degree of rigidity to the organ. Histologically the spleen is composed of splenic lobules. These are imperfectly defined by the interlobular trabeculae. Branching from these interlobular trabeculae the intralobular trabeculae divide the splenic lobule into about ten primary compartments. The space between this frame-work of trabeculae is filled by splenic pulp and lymph follicles. The splenic pulp is composed of essentially four elements namely, lymphocytes, leucocytes, erythrocytes and the large phagocytic cells.

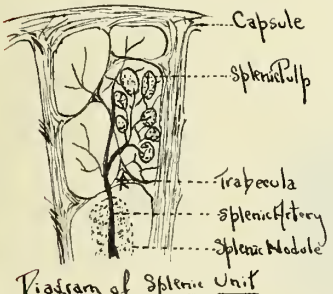


Diagram of Splenic Unit

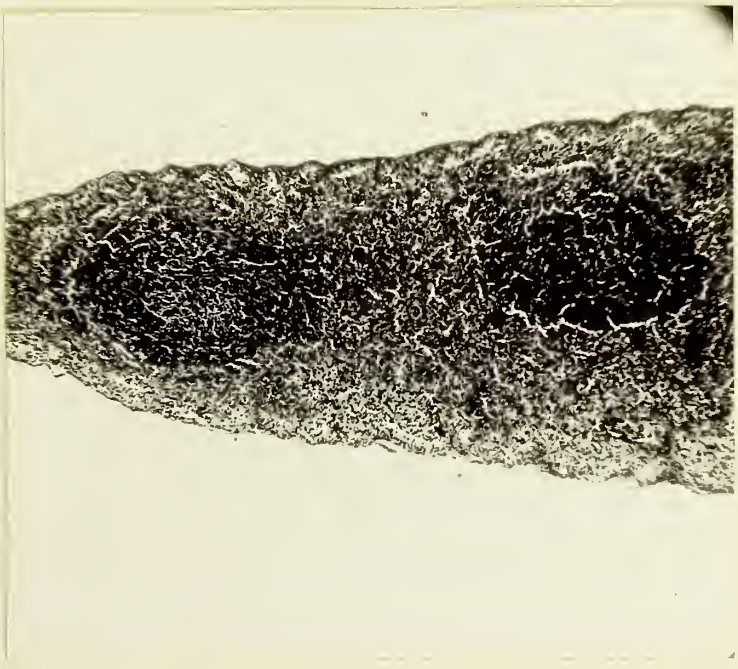
The blood enters at the hilum and after traversing in the hilum connective tissue for a short distance the arteries break up into branches which supply individual splenic lobules. Prolongations of the connective tissue of the trabeculae follow the arterial branches into the pulp, thus surrounding them with an extra fibrous envelope. Between this envelope and the artery wall local accumulations of lymphocytes occur giving rise to a spherical or fusiform mass of lymphoid tissue, the Malpighian bodies. The functional blood supply after con-

tinuing in vessels for a time, after entering at the hilum, a part leaves the vessels and flows freely thru the splenic pulp, while another part continues thru thin walled venous sinuses. There is a slow movement of the blood thru the spleen after which it is again collected in vessels and carried away. The nutritional blood supply on the other hand is conducted thruout its course in closed vessels.

SPLEEN

Histo-Pathological Changes:

Follicular hyperplasia was the outstanding feature of over 85% of the spleens. Most of the cases, however, were mild and only two were marked. Congestion was present in 65% of the cases. In one spleen there was an increased number of leucocytes while three others had eosinophilic infiltrations. A mild splenitis was found in one specimen.



Spleen--Histo-Pathological Changes

The increase in the size of the splenic follicles and the congestion were the most outstanding changes.

THE LUNGS

Normal Histology:

The lungs, the respiratory organs of the body, are composed of thin walled air sacs surrounded by thin walled capillaries which allow the air to come in close contact with the blood and permit a gaseous exchange.

On examining the outer surface of the lung it is seen that it is divided into polygonal areas, or lobules which are the unit of structure of the lung. Beginning at the trachea the air passages divide as seen in the figure below. The trachea extends well down into the thorax and then divides into two bronchi, one going to each lung. The bronchus on entering the lung begins to divide and subdivide. The branches are called bronchioles. The larger bronchioles have cartilaginous rings as in the walls of the main bronchi. The medium sized bronchioles contain only scattered plates of cartilage. These smaller bronchioles divide and finally end in terminal bronchioles. The terminal bronchioles are connected with the alveoli or air sacs by means of the atria.

On cross section the lung appears as shown in figure below. The walls of the air sacs are composed of flattened spherical cells. The blood supply follows the divisions of the bronchioles and extends into the interstitial tissue between the air sacs.

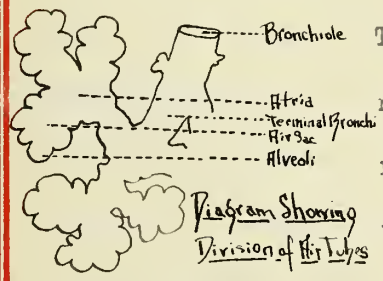


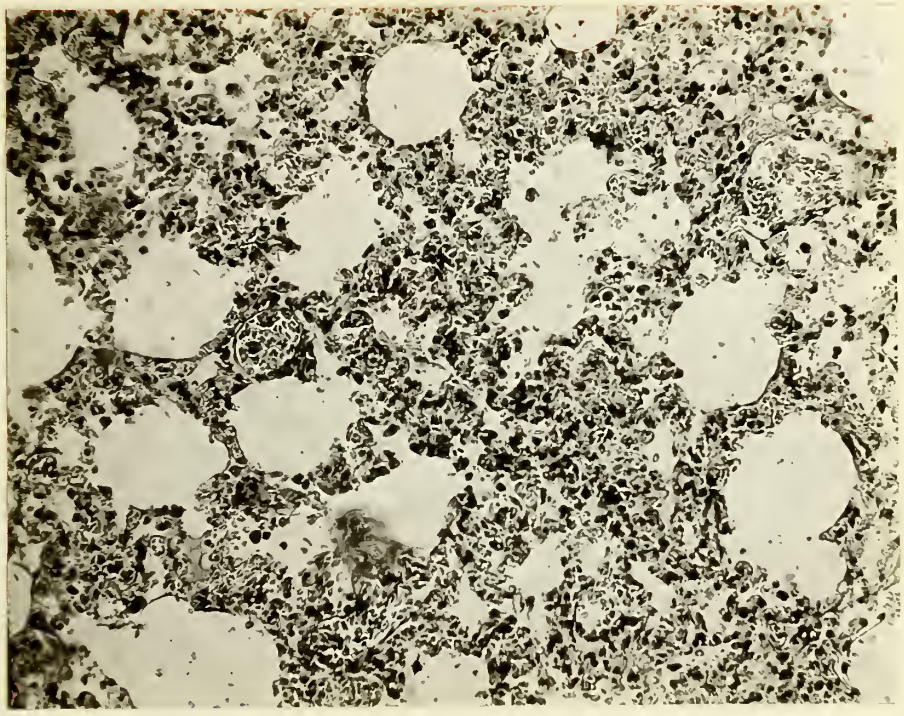
Diagram Showing Division of Air Tubes

The walls of the bronchioles are composed of three parts, namely, the fibrous tunic which contains the cartilaginous rings, the central layer of smooth muscular and connective tissues, and the mucous membrane lining the inner surface of the lumen.

THE LUNGS

Histo-Pathological Changes:

Passive congestion of the lungs was present in all of the cases but one. Three of the lungs apparently showed hemorrhages, which might be the result of agonal struggling in those few cases destroyed by concussion. Seven of the lungs were severely congested and six only slightly congested. One lung was normal.



Lung--Histo-Pathological Changes

A slight catarrhal exudate was present on the edges of the air sacs in one lung and another had a catarrhal bronchial exudate. Five lungs presenting advanced passive congestion also showed sub-pleural edema.

SUMMARY

The most consistent histo-pathological change in the kidney, liver, heart, spleen and lung of guinea pigs suffering from an acute form of botulinus poisoning was a passive congestion, with parenchymatous degeneration in varying degrees. The later in a few instances bordered on necrosis. Occasionally beginning inflammatory changes were encountered, but in the series as a whole this was not characteristic. Similar changes may be observed in other toxemic diseases, and are of little or no value in rendering a differential diagnosis. The acute character of the disease in the guinea pigs that furnished the tissues for these studies may not be typical of changes in a chronic form of botulinus intoxication.

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DIAGNOSIS CHART OF KIDNEYS

No.	Blood	Cloudy Swelling	Remarks
8-1-A	Congestion-Localized hem* Slight hem.in glomeruli.	C.S**in varying degrees. Some border on necrosis	Slight edema
9-1-A	Passive Congestion. Slight cong.in glom#	Marked c.s.of T.***	Collection of red cells under capsule°Slight necrosis at hem.
13-B	Severe passive cong.	Marked c.s.of T.	
14-c	Passive Congestion	Marked c.s.of T.	
15-C	Very severe cong.	Marked c.s.of T.	
16-c	Slight pass.cong.	Mild c.s.of T.	
17-2-A	Severe pass.cong.	Mild c.s.of T.	
18-1-A	Severe pass.cong.	Marked c.s.of T.	Areas of necrosis
19-A	Severe pass.cong.	C.s.of T.	
21-1-A	Beginning pass.cong.	Slight c.s.of T.	
23-2-A	Severe pass.cong.	Severe c.s.of T.	
24-A		Marked c.s.of T.	
25-A	Severe pass.cong.	Slight c.s.of T.	
26-C		Slight c.s.of T.	
27-B		Slight c.s.of T.	
28-B	Marked pass.cong.	Marked c.s.of T.	
29-A	Slight pass.cong.	Slight c.s.of T	
30-D	Slight pas.cong.	Slight c.s.of T.	
31-A	Marked pas.cong.	Slight c.s.of T.	

*Hemorrhage C.s.**Cloudy Swelling of Urinary Tubules
#Glomeruli.

DIAGNOSIS CHART OF THE LIVERS

No.	Blood	Cloudy Swelling	Degenerations	Remarks.
8	Severe pass cong.	Slight c.s.*	Fatty infilt.& degeneration	Slight cellular hepatitis.
13-A	Pass.cong.	Severe c.s.	Fatty infilt & deg.(slight)	
14-D	Slight p.c**	Severe c.s.	Slight fatty deg.	
15-B	Marked p.c.	Slight c.s.	Slight fatty deg.	
16-B	Slight p.c.	C.s.	Severe fatty deg.	Slight cellular hepatitis.
17-2-C	Mild p.c.	C.s.	Fatty deg.& Infilt.	
18-1-C	Slight p.c.	Marked c.s.	Fatty deg.	Deg.near necrosis
19-C	Slight p.c.		Marked fatty deg.	Slight cellular hepatitis
21-C			Marked fatty deg.	
23-1-C	Severe p.c.	Marked c.s.	Marked fatty deg.	
24-C		Slight c.s.	Severe fatty deg.	Slight cellular hepatitis
25-D	Marked p.c.	Marked c.s.	Marked fatty deg.	
26-B			Mild fatty deg.	
27-C	Mild p.c.	Mild c.s.	No fatty deg.	
28-E		Marked c.s.	Fatty infilt.on piripheral areas	
29-A	Slight p.c.	Slight c.s.		
30-B	Slight p.c.	Slight c.s.	Marked fatty deg.	
31-D	Slight p.c.	Marked c.s.	Slight fatty deg.	

c.s*cloudy swelling **Passive Congestion

DIAGNOSIS CHART OF THE HEARTS

No.	Blood	Cloudy Swelling	Remarks
13-c-1		Striations not visible due to c.s.*	
14-B		Marked c.s. All striations gone	
15-A	Cong. of coronary vessels	Severe c.s.	
17-1-A	Pass. cong. of capillaries of cardiac muscle.	Marked c.s. Striations gone	
18-1-B		Slight c.s. Striations gone	Slight separation at cement lines° Fibers shrunken.
21-B		Mild c.s.	
23-1-A		Very slight c.s.	Slight separation at cement lines.
24-B	Coronary vessels congested	Striations visible in part of the fibers	Separation at cement lines.
25-B	Cong. of coronary vessels.	Slight c.s.	
26-E		Very mild c.s.	
27-A		Slight c.s.	
28-D		Slight c.s.	
29-D	No congestion	Very little c.s.	
30-C		Slight c.s.	
31-C	Cong. of coronary vessels	Slight c.s.	

*Cloudy Swelling

DIAGNOSIS CHART OF THE SPLEENS

No.	Blood	Follicular Hyperplasia	Remarks
9-1-C	General cong. Increase in leuk*	Mild f.h.**	
13-F	Congestion		
14-F		f.h.	
15-2-A		Slight f.h.	
16-E	No congestion	Slight f.h.	
17-1-B		Slight f.h.	
18-2-B	Mild congestion		
19-E	Slight cong.	Slight f.h.	
21-3-B			Mild interstitial splinitis
23-2-C	Slight cong.	f.h.	
24-F	Congestion	f.h.	
26-F			Focal collections of eosinphiles.
27-F		f.h.	Eosinphilic infiltration
28-F	Very slight cong.	Slight f.h.	Increase of eosinphiles
29-F	Very slight cong.	Marked f.h.	
31-E		Mild f.h.	

*Leucocytes **Follicular Hyperplasia

DIAGNOSIS CHARTS OF THE LUNGS

No.	Blood	Exudate	Remarks
8-1-D	Passive cong*	Slight catarrhal exudate on the edges of air sacs.	
9-2-D	Very severe passive congestion		Mild compensatory emphysema
13-E	Slight congestion		
14-A	Severe cong.in most areas?Hem.over a large area.		Sub-Pleura edema A few eosinphiles.
15-D	Slight passive cong.		Suggestive of emph ysema.
16-A	Slight passive cong.		
17-1-E	Severe passive cong.		Slight sub-pleura edema.
18-1-D	Severe passive cong.		
19-D	Slight passive cong.		
21-2-A	Hypostatic cong.		Desquamation of epth. cells of air sacs.
23-1-B	Very severe cong.		Edema-Desquamation of alveloir cells.
24-1-E	Marked congestion		Slight edema
25-C	Very severe cong.		
26-A	Slight congestion		
27-E	Normal		
28-A	Hemorrhage		
29-E	Very marked cong.	Slight exudate in bronchi	
30-A	Slight passive cong.		
31-B	Hemorrhage		Edema-Blood in bronchi.

*Congestion